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Effects of mycorrhizal roots and extraradical hyphae on ^{15}N uptake from vineyard cover crop litter and the soil microbial community

Xiaomei Cheng[a], Kendra Baumgartner[b,*]

[a]*Department of Plant Pathology, University of California, Davis, CA 95616, USA*
[b]*US Department of Agriculture, Agricultural Research Service, Department of Plant Pathology, University of California, one Shields Avenue, Davis, CA 95616, USA*

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Abstract

The objectives of this study were to evaluate the contribution of arbuscular mycorrhizal (AM) fungal hyphae to ^{15}N uptake from vineyard cover crop litter (*Medicago polymorpha*), and to examine the soil microbial community under the influence of mycorrhizal roots and extraradical hyphae. Mycorrhizal grapevines (*Vitis vinifera*) were grown in specially designed containers, within which a polyvinyl chloride (PVC) mesh core was inserted. Different sizes of mesh allowed mycorrhizal roots (mycorrhizosphere treatment) or extraradical hyphae (hyphosphere treatment) to access dual labeled ^{15}N and ^{13}C cover crop litter that was placed inside the cores after 4 months of grapevine growth. Mesh cores in the bulk soil treatment, which served as a negative control, had the same mesh size as the hyphosphere treatment, but frequent rotation prevented extraradical hyphae from accessing the litter. Grapevines and soils were harvested 0, 7, 14, and 28 days after addition of the cover crop litter and examined for the presence of ^{15}N. Soil microbial biomass and the soil microbial community inside the mesh cores were examined using phospholipid fatty acid analysis. ^{15}N concentrations in grapevines in the hyphosphere treatment were twice that of grapevines in the bulk soil treatment, suggesting that extraradical hyphae extending from mycorrhizal grapevine roots may have a role in nutrient utilization from decomposing vineyard cover crops in the field. Nonetheless, grapevines in the mycorrhizosphere treatment had the highest ^{15}N concentrations, thus highlighting the importance of a healthy grapevine root system in nutrient uptake. We detected similar peaks in soil microbial biomass in the mycorrhizosphere and hyphosphere treatments after addition of the litter, despite significantly lower microbial biomass in the hyphosphere treatment initially. Our results suggest that although grapevine roots play a dominant role in the uptake of nutrients from a decomposing cover crop, AM hyphae may have a more important role in maintaining soil microbial communities associated with nutrient cycling.
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Keywords: Arbuscular mycorrhizae; Decomposition; Hyphosphere; *Medicago polymorpha*; Mycorrhizosphere; N uptake; PLFA; *Vitis vinifera*

1. Introduction

Arbuscular mycorrhizal (AM) fungi benefit grapevines (*Vitis vinifera*) in terms of improved shoot and root growth (Schubert et al., 1988; Biricolti et al., 1997; Linderman and Davis, 2001), higher tissue concentrations of P (Biricolti et al., 1997), and the production of a more compact, highly branched root system (Schellenbaum et al., 1991). In the field, AM fungi naturally colonize grapevine roots, as demonstrated by studies on indigenous AM fungi in California vineyards (Menge et al., 1983; Cheng and Baumgartner, 2004b) and in other grape-growing regions (Possingham and Groot-Obbink, 1971; Deal et al., 1972; Nappi et al., 1985). When AM fungi do not naturally colonize grapevine roots, such as after soil fumigation, they may suffer severe nutrient deficiencies (Menge et al., 1983). The results of these studies collectively support the hypothesis that AM fungi play essential roles in grapevine nutrition.

Vineyard cover crops have been shown to enhance indigenous populations of AM fungi in vineyard soils and grapevine roots (Baumgartner et al., 2005; Cheng and Baumgartner, 2005). Vineyard cover crops host AM fungi, with the notable exception of *Brassica* species (e.g. *B. nigra* (L.) Koch) (Schreiner and Koide, 1993). In California,

*Corresponding author. Tel.: +1 530 754 7461; fax: +1 530 754 7195.
E-mail address: kbaumgartner@ucdavis.edu (K. Baumgartner).

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planting cover crops in between vineyard rows is a common practice during the dormant season, used mainly to reduce soil erosion from winter rains, and also to improve soil fertility and structure (Ingels et al., 1998). Overlap of grapevine and cover crop roots may encourage interactions among grapevines, cover crops, and AM fungi, such as the formation of common mycorrhizal networks.

Previously, we found evidence of direct nutrient transfer from cover crops to grapevines via AM fungal links (Cheng and Baumgartner, 2004a). N transfer was significantly greater from the grass, *Bromus hordeaceus* L. ssp. *molliformis* (Lloyd) Maire & Weiller cv. Blando, to the grapevine than from the legume, *Medicago polymorpha* L. cv. Santiago, to the grapevine. Our findings demonstrated that certain cover crop species may be better than others at enhancing AM fungi-mediated nutrient transfer from cover crops to grapevines, possibly by hosting different AM fungal species or by supporting a greater root biomass.

Cover crop management practices, specifically mowing and tilling, may also affect AM fungi-mediated nutrient transfer from vineyard cover crops to grapevines. In spring, no-till cover crops are mowed and their root systems remain intact, whereas the shoots and roots of tilled cover crops are incorporated into the soil. Grapevines have been shown to utilize N from cover crops soon after tilling (Patrick et al., 2004). Extraradical hyphae extending from mycorrhizal roots may absorb nutrients from decomposing cover crops, given their abilities to accelerate decomposition and acquire N directly from complex organic materials, such as grass shoots (Hodge et al., 2001). In addition, they can proliferate organic materials and recycle mineralized nutrients efficiently, thereby improving host nutrient uptake from decomposed organic materials (St John et al., 1983; Aristizábal et al., 2004). Therefore, grapevines with higher mycorrhizal colonization may have a greater capacity to utilize nutrients from decomposing cover crop material than grapevines with minimal root colonization.

AM fungi may affect other soil microbes associated with decomposition of cover crops, given that their extraradical hyphae distribute photosynthesis-derived carbon (C) to the soil and, thus, contribute to the labile pool of C that other soil microbes utilize. The AM fungal hyphosphere, the zone of soil affected by the extraradical hyphae (Marschner, 1995), may support a distinct microbial community within the mycorrhizosphere, the zone of soil affected by both the root and hyphal components of a mycorrhiza (Linderman, 1988). Distinctions in the microbial communities between the mycorrhizosphere and hyphosphere may be attributed to differences in C and other nutrients derived from plant roots and hyphae. Extraradical hyphae, which grow extensively in root-free soil and form networks between adjacent host plants, are the most dynamic and functionally diverse, but the most poorly understood, components of the symbiosis (Staddon et al., 2003; Leake et al., 2004). Their functional significance within the mycorrhizosphere and as part of the soil microbial community has long been ignored until recently with the application of advanced techniques (Leake et al., 2004), such as the use of isotope tracers and phospholipid fatty acid (PLFA) markers.

The goal of this study was to examine AM fungi-mediated nutrient uptake from a tilled vineyard cover crop, *M. polymorpha* L. cv. Santiago. Specific objectives were to: (1) evaluate the contribution of AM fungal hyphae, relative to that of grapevine roots, to ^{15}N uptake from the decomposing cover crop, (2) quantify the response of the soil microbial community to the decomposing cover crop under the influence of mycorrhizal roots (mycorrhizosphere effects) or hyphae (hyphosphere effects), and (3) examine the effects of AM fungal hyphae, relative to that of grapevine roots, on decomposition of the cover crop tissue.

2. Materials and methods

2.1. Experimental design

Grapevines used in this study were *V. vinifera* L. cv. Cabernet Sauvignon (ENTAV clone 338), grafted onto 110 R rootstock (*V. berlandieri* Planch. X *V. rupestris* Scheele). We utilized the same container and mesh core design as in our previous labeling study with grapevines and cover crops (Cheng and Baumgartner, 2004a), but in this experiment no cover crops were grown inside the mesh core. Grapevines were grown in containers (20 cm diameter × 25 cm depth) within which a polyvinyl chloride (PVC) mesh core was vertically inserted. Mesh cores, modified from a design by Johnson et al. (2001), were constructed by cutting four windows into a 25-cm-long section of PVC pipe (6.8 cm inner diameter, 7.2 cm outer diameter), which removed approximately 50% of its external surface. The inner and outer surfaces of each pipe were then wrapped with a layer of either 1 mm plastic mesh to allow grapevine roots to penetrate the mesh core, or 25-μm stainless-steel mesh to exclude roots. A piece of 2-mm-thick plastic mesh ($8 \times 7\,cm^2$) was inserted at each window between the two layers of 1-mm or 25-μm mesh, in order to create an air gap. Containers were randomly arranged on one greenhouse bench in a completely randomized design. The growth medium consisted of a 1:1 mixture of field soil (collected from a winegrape vineyard in Napa, CA, USA) and sterile sand. The soil and sand mixture contained 0.07% total N, $38\,\mu g\,g^{-1}$ of Olsen P, and $4\,\mu mol\,g^{-1}$ of exchangeable K with $102\,\mu mol\,g^{-1}$ of cation exchange capacity.

Dormant grapevines, obtained from a commercial grapevine nursery in California, were rooted in pots in the greenhouse. Dormant roots of field-propagated grapevines contain AM fungi that serve as inoculum for new roots (Cheng and Baumgartner, 2004b). To increase mycorrhizal colonization of the grapevines, chopped grapevine fine roots and rhizosphere soil, collected from the same vineyard we obtained field soil from, were added

to the soil and sand mixture as an additional source of inoculum. Each container received the same amount of inoculum (approximately 100 ml in volume), which was incorporated into the top 10 cm of growth medium in the pot (both inside and outside of the mesh core) 1 day after grapevines were rooted. Plants were fertilized weekly with Hoagland's nutrient solution (Epstein, 1972) with 0.25 strength of the standard P concentration. After 2 months, grapevine shoots, which were all extremely vigorous, were pruned to two shoots per grapevine.

At the time of planting the grapevines, a mesh core was vertically inserted inside each container and was filled with the same soil that the grapevines were planted in. A very narrow PVC pipe (2.1 cm diameter × 25 cm length) was vertically inserted in the soil at the center of each mesh core, to a depth of 20 cm, to reserve a column of space for the eventual addition of the labeled cover crop litter. There were three mesh core treatments: 1-mm plastic mesh was used to allow fine grapevine roots and AM fungal hyphae to penetrate the mesh core (mycorrhizosphere treatment); 25-μm stainless-steel mesh was used to allow only hyphae to penetrate the mesh core (hyphosphere treatment); and 25-μm stainless-steel mesh was used to exclude roots, and the mesh core was rotated within the containers every other day to exclude hyphae (bulk soil treatment). There were a total of 16 grapevines per mesh core treatment.

2.2. Labeling experiment

The cover crop, *M. polymorpha* L. cv. Santiago (a legume), was grown in 3.8-L containers in potting mix, which consisted of a 1:1:1 ratio of peat moss, perlite, and Supersoil® (Rod McClellan Co., South San Francisco, CA, USA) by volume. After 4 weeks of growth, cover crops were watered with 16 mM $K^{15}NO_3$ solution weekly for a total of 4 weeks. After 5 and 7 weeks, cover crops were pulse-labeled with ^{13}C (99 at% of $^{13}CO_2$) for 1 day using a labeling chamber. After 8 weeks, cover crop shoots were harvested, oven dried, finely ground using a Wiley mill (passed through a 1-mm mesh screen), and analyzed for ^{13}C and ^{15}N enrichment (Stable Isotope Facility, University of California, Davis, CA, USA). The dried cover crop shoots contained 3.37% of total N, with 3.55 at% of ^{15}N enrichment and 41.5% of total C, with 1.54 at% ^{13}C enrichment.

The cover crop litter consisted of 5 g of dried cover crop shoots plus 70 g of the soil and sand growth media. After 4 months of grapevine growth, the narrow PVC pipe was removed from the center of each mesh core and labeled cover crop litter was carefully poured inside the empty column of space. This created a 20-cm-long, cylindrical column of labeled cover crop litter at the center of each mesh core (positioned at a soil depth of 0–20 cm). Approximately 20 ml of water was then slowly added into the mesh cores to moisten the cover crop litter.

Grapevines were destructively sampled for leaf, stem, and root tissues at days 0, 7, 14, and 28 (four replicates per sampling date per mesh core treatment), after adding the cover crop litter to the mesh cores. Tissues were dried at 70 °C for 7 days, weighed, and analyzed separately for total N and ^{15}N with a mass spectrometer (Stable Isotope Facility, University of California, Davis, CA, USA). Roots that grew within the mesh cores of the mycorrhizosphere treatment were manually picked from the soil and cover crop litter, dried at 70 °C for 7 days, and weighed.

On each sampling date, when grapevines were destructively sampled, container soil from outside the mesh cores (where the grapevines were planted) was homogenized and subsamples were collected for ^{15}N analysis. The column of cover crop litter that occupied the center of each mesh core remained intact throughout the experiment; it was visually distinguishable from the surrounding soil. This column was carefully removed and analyzed for ^{13}C and ^{15}N. We collected soil samples from the area immediately surrounding the cover crop litter, in between the inner wall of the mesh core and the cover crop litter, and stored them at −20 °C until PLFA analyses. A subsample of the soil collected on the day we planted the grapevines was also stored at −20 °C until PFLA analyses. Phospholipids were extracted, fractionated, methylated, and analyzed by gas chromatography as described in Bossio and Scow (1998). Individual PLFAs were identified using the bacterial fatty acid standards and identification software from a microbial identification system (Microbial ID, Newark, DE, USA). Fatty acids were quantified by comparing the individual PLFA peak areas with that of the internal standard, 19:0. The total extractable PLFA provided a measure of soil microbial biomass, expressed as nmol/g dry soil.

Fatty acid terminology utilizes '$A:B\omega C$' where 'A' indicates the total number of C atoms, 'B' indicates the number of unsaturations, and 'ω' precedes 'C', the number of C atoms between the closest unsaturation and the aliphatic end of the molecule. The suffixed 'c' and 't' indicate *cis* and *trans* geometric isomers. The prefixed 'I' and 'a' refer to *iso* and *anti-iso* methyl branching. Hydroxy groups are indicated by 'OH' and cyclopropyl groups by 'cy'. '10Me' refers to a methyl group on the tenth C from the carboxylic end of the fatty acid. Individual fatty acids were classified as indicators for four specific soil microbial groups: actinomycetes: i17:1 (Vestal and White, 1989), 10Me16:0, 10Me17:0 (O'Leary and Wilkinson, 1988), 10Me18:0 (Kroppenstedt, 1985); Gram +ve bacteria: i14:0 (Federle, 1986; Zelles, 1997), i15:0, a15:0, i16:0, i17:0, a17:0 (Zelles, 1997); Gram −ve bacteria: 16:1ω7t, 16:1ω7c (Zelles, 1997), 17:1ω9c (O'Leary and Wilkinson, 1988), cy17:0 (Zelles, 1997), cy19:0 (Federle, 1986; Zelles, 1997); and fungi: 16:1ω5c (Olsson et al., 1995), 18:3ω6, 9, 12c (Vestal and White, 1989; Zelles, 1997), 18:2ω6, 9c (Federle, 1986; Zelles, 1997), 18:1ω9c (O'Leary and Wilkinson, 1988). Remaining fatty acids (3OH14:0, 12:0, 14:0, i15:1, 15:0, 16:1ω11c, 16:0, i17:1ω5c, 17:0, 16:12OH, 18:0, cy19:0ω10c, 20:0) were listed as unspecific soil microbes. Fatty acid 16:1ω5c, identified as an AM fungal biomarker in a previous study (Olsson et al, 1995), was

analyzed separately, in order to measure AM fungal biomass inside the mesh cores of our three treatments.

2.3. Calculations and statistical analyses

N transfer was estimated based on the assumption that equal proportions of labeled and nonlabeled N were transferred. Percentage of cover crop litter N transferred to grapevines (%N transfer) and percentage N in the grapevine derived from cover crop litter (%NDFL) were calculated using the following formulas:

$$\%N\ \text{transfer} = \frac{\text{total } {}^{15}\text{N}_{\text{grapevine}}}{\text{total } {}^{15}\text{N}_{\text{cover crop litter}}} \times 100,$$

$$\%\text{NDFL} = \frac{\%\ N\ \text{transfer} \times \text{total } N_{\text{cover crop litter}} \times 100}{\text{total } N_{\text{grapevine}}},$$

where total ^{15}N of cover crop litter = 5.95 mg and total N of cover crop litter = 168.6 mg.

Data were analyzed using the MIXED procedure in SAS (SAS System, version 8.2, SAS Institute, Cary, NC, USA). A three-way ANOVA was used to examine the effects of mesh core treatment and sampling date on grapevine biomass, N concentration, and ^{15}N at% excess in grapevine tissues (mesh core treatment, sampling date, and grapevine tissue as main factors). A separate three-way ANOVA was used to examine the effects of mesh core treatment and sampling date on ^{15}N at% excess in grapevines in the hyphosphere and bulk soil treatments. A two-way ANOVA was used to examine the effects of mesh core treatment and sampling date on grapevine total ^{15}N, %N transfer, %NDFL, soil ^{15}N and ^{13}C, total soil microbial biomass, the biomass of the five soil microbial groups, and fatty acid 16:1ω5c. All factors were treated as fixed effects. To normalize variances, a square root transformation was performed on grapevine total ^{15}N, %N transfer, and %NDFL, and a log transformation was performed on grapevine biomass, total soil microbial biomass, and fungal biomass. A simulation-based method was used to obtain critical values for treatment mean comparisons, using the ADJUST = SIMULATE option for the LSMEANS statement in SAS (Edwards and Berry, 1987). Correspondence analysis (CANOCO, version 4.5, Microcomputer Power, Inc., Ithaca, NY) was used to determine the relationships among samples from the different mesh core treatments and sampling dates, based on the soil microbial fatty acids present and their relative abundances. Only those fatty acids present in 30% or more of the samples were included in the analysis.

3. Results

3.1. ^{15}N in grapevine tissues

There were significant differences in ^{15}N at% excess in grapevines among the mesh core treatments and sampling dates (Table 1). ^{15}N at% excess increased over time in

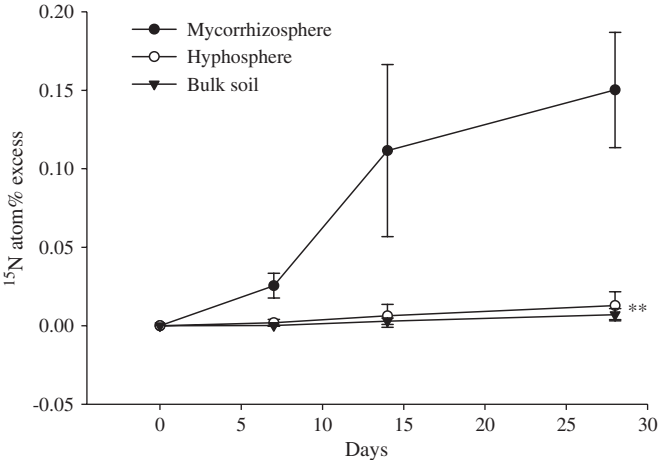

Fig. 1. Effect of mesh core treatment on ^{15}N at% excess in grapevines before (day 0) and 7, 14, and 28 days after adding labeled cover crop litter to the mesh cores. Error bars represent standard deviations. Each point is the mean of 12 observations summed over grapevine tissues (leaves, stems, and roots). **, a significant difference was found between the hyphosphere and bulk soil treatments, based on results of ANOVA restricted to these treatments ($P = 0.0028$, simulation-based t-test).

Table 1
F values from analyses of variance for ^{15}N atom% excess in grapevines with three mesh core treatments (mycorrhizosphere, hyphosphere, bulk soil), and two mesh core treatments (hyphosphere, bulk soil)

Source[a]	Three mesh core treatments		Two mesh core treatments	
	F	$P > F$	F	$P > F$
Tissue	3.95	**0.0303**	7.31	**0.0025**
Treatment	148.87	**<0.0001**	10.50	**0.0028**
Tissue × treatment	3.98	**0.0079**	0.70	0.5024
Sampling date	57.96	**<0.0001**	21.74	**<0.0001**
Tissue × sampling date	1.36	0.2706	1.10	0.3752
Treatment × sampling date	20.86	**<0.0001**	0.92	0.4091
Tissue × treatment × sampling date	0.94	0.4942	0.06	0.9920

Significant P values ($P \leqslant 0.05$) are shown in bold.
[a] *Source of variation*: Tissue—grapevine leaves, stems, or roots; Mesh core treatment—mycorrhizosphere, hyphosphere, or bulk soil; Sampling date—day 0, day 7, day 14, or day 28.

grapevines in all three mesh core treatments (Fig. 1). ^{15}N at% excess in the mycorrhizosphere treatment, in which both roots and hyphae were allowed to access labeled cover crop litter in the center of mesh core, was significantly higher than in the hyphosphere and bulk soil treatments, especially from day 7 to day 14 (Fig. 1). In the bulk soil treatment, in which both roots and hyphae were excluded from the mesh core, ^{15}N at% excess was significantly higher in the roots than in the leaves (Fig. 2). In the mycorrhizosphere treatment, in contrast, ^{15}N at% excess was lower in the roots than in the leaves and stems (Fig. 2), hence the significant treatment × tissue interaction (Table 1).

Grapevines in the mycorrhizosphere treatment took up disproportionately higher amounts of ^{15}N (Fig. 1); therefore, we conducted a separate ANOVA of mesh core treatment effects on ^{15}N at% excess in grapevine tissues that was restricted to comparisons between the hyphosphere and bulk soil treatments (Table 1). All main effects were significant and were not confounded by interaction effects, in contrast to the results of the ANOVA among all three mesh core treatments (Table 1). Grapevines in the hyphosphere treatment had significantly higher ^{15}N at% excess in all tissues (leaves, 0.0044%; stems, 0.0056%; roots, 0.0109%), compared to grapevines in the bulk soil treatment (leaves, 0.0022%; stems, 0.0031%; roots, 0.0053%).

When fine roots were allowed to access the mesh core in the mycorrhizosphere treatment, grapevine total ^{15}N, %N transfer, and %NDFL were significantly higher than when roots were excluded in the hyphosphere and bulk soil treatments, in all cases (Table 2). For example, on day 28, total ^{15}N of grapevines in the mycorrhizosphere treatment (927 μg) was 15 times higher than that of grapevines in the hyphosphere treatment (62 μg). In the hyphosphere treatment, total ^{15}N, %N transfer, and %NDFL were higher than in the bulk soil treatment, but these differences were not significant (Table 2).

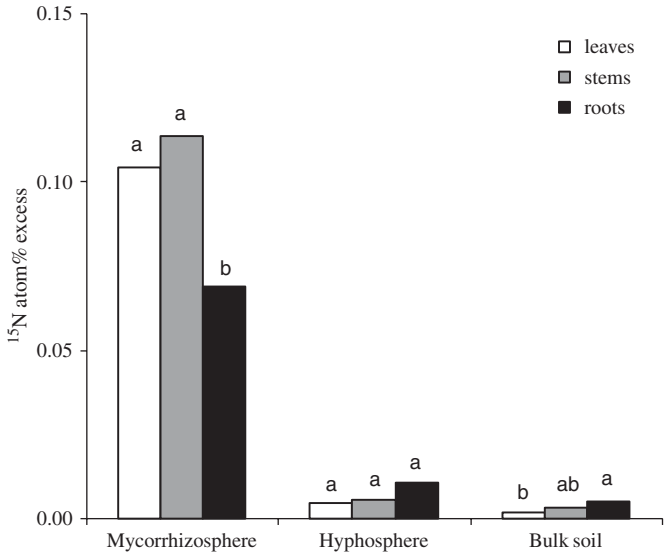

Fig. 2. Effect of mesh core treatment on ^{15}N at% excess in grapevine tissues. Each column is the mean of 12 observations summed over sampling dates. Columns with different letters are significantly different at $P \leqslant 0.05$ (simulation-based t-test) within each mesh core treatment.

3.2. Grapevine tissue biomass and N concentration

There were significant differences in grapevine biomass among the mesh core treatments ($P < 0.0001$). Grapevines in the bulk soil treatment had 20–23% lower biomass (48.59 g) than grapevines in the hyphosphere treatment (57.96 g) and the mycorrhizosphere treatment (59.87 g). Stem and leaf biomass did not change significantly over time. However, root biomass increased significantly from day 0 (13 g) to day 7 (22 g). There were no significant differences in N concentration of grapevine tissues among mesh core treatments or sampling dates, although N concentration was higher in the leaves, 2.98%, than in the roots, 1.09%, and stems, 0.63%.

The fine mesh used in the bulk soil and hyphosphere treatments prevented grapevine roots from growing within the mesh cores. As expected, roots were present within every core of the mycorrhizosphere treatment, but increases in their biomass over time, 0.52 g on day 0 to 0.71 g on day 28, were not significant ($P = 0.2030$). There were no significant differences in mycorrhizal colonization of grapevine roots among mesh core treatments or sampling dates ($P = 0.9254$ and 0.1651, respectively). The mean mycorrhizal colonization of all samples was 77.4% of root length.

Table 2
Total ^{15}N derived from the cover crop litter in grapevines, percentage of cover crop litter N transferred to grapevines (%N transfer), and percentage of N in grapevine derived from litter (%NDFL)

Mesh core treatment	Total ^{15}N (μg)			%N transfer			%NDFL		
	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28	Day 7	Day 14	Day 28
Mycorrhizosphere	154.98a	681.40a	927.40a	2.61a	11.49a	15.64a	0.73a	3.23a	4.27a
Hyphosphere	9.94b	32.31b	62.48b	0.17b	0.54b	1.05b	0.05b	0.17b	0.35b
Bulk soil	0.00b	12.26b	36.40b	0.00b	0.21b	0.61b	0.00b	0.08b	0.21b

Means ($n = 4$) followed by different letters in the same column are significantly different at $P \leqslant 0.05$, simulation-based t-test.

3.3. ^{15}N and ^{13}C in the cover crop litter

Both ^{15}N at% and ^{13}C at% decreased significantly ($P<0.0001$) in the cover crop litter from day 0 (2.8% ^{15}N, 1.5% ^{13}C) to day 28 (2.0% ^{15}N, 1.3% ^{13}C). Decomposition rates were highest the first week after addition of the cover crop litter. The effects of mesh core treatment on ^{15}N at% and ^{13}C at% were not significant ($P = 0.4234$ and 0.3691, respectively). Changes in total N and C in the cover crop litter showed the same pattern over time as their corresponding at% data (*data not shown*).

There was slight ^{15}N enrichment of the soil outside the mesh cores. ^{15}N at% excess increased significantly in the soil from undetectable levels on day 0 to detectable levels on day 28 ($P = 0.0076$). By day 28, ^{15}N at% excess in the soil outside the mesh cores was still quite low; values ranged from 0.001% to 0.004%. ^{15}N at% excess was higher in the soil outside the mesh cores in the mycorrhizosphere treatment, 0.002–0.004%, than in the hyphosphere and bulk soil treatments, which were not significantly different from each other, 0.001%.

3.4. Soil microbial biomass and community inside the mesh cores

There was a significant effect of the interaction of mesh core treatment and sampling date on total microbial biomass ($P = 0.0073$) in soil within the mesh cores. Before addition of the cover crop litter, on day 0, total microbial biomass was significantly greater in the mycorrhizosphere treatment than in the hyphosphere and bulk soil treatments (Fig. 3). After addition of the cover crop litter, on day 7, total microbial biomass increased significantly, and to similar levels, in both the mycorrhizosphere and hyphosphere treatments. In contrast, total microbial biomass in the bulk soil treatment did not change significantly over time.

Regardless of mesh core treatment or sampling date, total microbial biomass consisted primarily of nonspecific microbes and fungi, which represented 29–34% and 23–30% of the total biomass, respectively (*data not shown*). There were significant interaction effects of mesh core treatment and sampling date on biomass of the five microbial groups (actinomycetes, $P = 0.0065$; Gram +ve bacteria, $P = 0.0035$; Gram −ve bacteria, $P = 0.0195$; fungi, $P = 0.0128$; nonspecific microbes, $P = 0.0076$). After addition of the cover crop litter, on day 7, biomass of each microbial group increased in the mycorrhizosphere and hyphosphere treatments, but not in the bulk soil treatment (Table 3). Most day 7 peaks in biomass were significant in the hyphosphere treatment. In the mycorrhizosphere treatment, on day 28, biomass of Gram +ve bacteria and Gram −ve bacteria reached levels that were intermediate between original levels and day 7 peaks. In the hyphosphere treatment, in contrast, day 7 peaks in biomass of Gram +ve bacteria, Gram −ve bacteria, fungi, and nonspecific microbes, all of which were significantly higher after addition of the cover crop litter, were followed by a return to original levels.

The biomass of fatty acid 16:1ω5c in a subsample of the growth medium that was collected on the day the grapevines were planted was 0.33 nmol g^{-1}, which represents 55% of that detected in the mycorrhizosphere treatment on day 0, and 89% of that detected in the hyphosphere and bulk soil treatments on day 0. There were significant main effects ($P<0.0001$) and interaction effects ($P = 0.0086$) of mesh core treatment and sampling date on the biomass of fatty acid 16:1ω5c. The biomass of 16:1ω5c was greatest in the mycorrhizosphere treatment, and greater in the hyphosphere than in the bulk soil treatments ($P = 0.0027$). The biomass of 16:1ω5c increased with addition of cover crop litter on day 7 in both the mycorrhizosphere and hyphosphere treatments. There were no significant changes in the biomass of 16:1ω5c in the bulk soil treatment over time.

Correspondence analysis of fatty acid fingerprints showed that the first and second axes explained 37.5% and 18.9%, respectively, of the variation in the microbial community among samples. The first axis appears to be

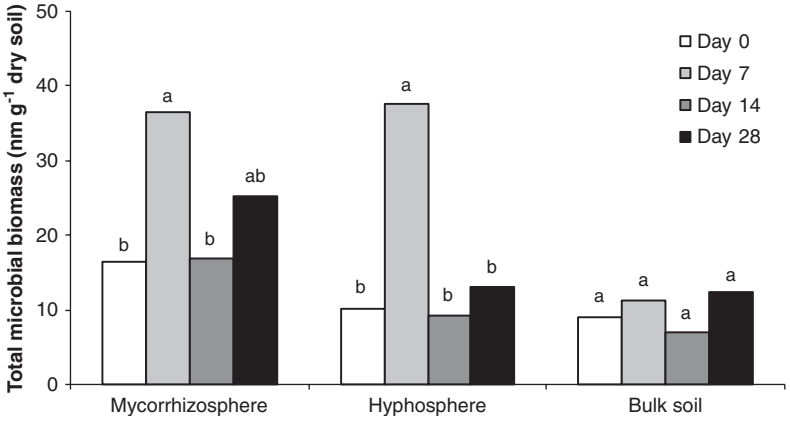

Fig. 3. Effects of three mesh core treatments and four sampling dates on total microbial biomass. Columns with different letters are significantly different at $P \leqslant 0.05$ (simulation-based t-test) within each mesh core treatment.

Table 3
Effects of mesh core treatments and sampling dates on the biomass of actinomycetes, Gram +ve bacteria, Gram −ve bacteria, fungi, nonspecific microbes, and fatty acid 16:1ω5c

Treatments	Days	Biomass (nm g^{-1} dry soil)					
		Actinomycetes	Gram +ve bacteria	Gram −ve bacteria	Fungi	Nonspecific microbes	16:1ω5c
Mycorrhizosphere	0	1.19a	2.78b	1.86b	5.18a	5.40a	0.60ab
	7	1.73a	6.99a	6.02a	11.33a	10.45a	0.82a
	14	0.94a	3.37b	2.71b	4.78a	5.13a	0.38b
	28	1.69a	4.64ab	3.35ab	7.91a	7.74a	0.67ab
Hyphosphere	0	0.92ab	2.08b	1.20b	2.49b	3.52b	0.38ab
	7	2.02a	7.65a	5.72a	11.19a	10.78a	0.73a
	14	0.57b	1.96b	1.30b	2.36b	2.99b	0.22b
	28	0.94ab	2.61b	1.78b	3.54b	4.19b	0.55a
Bulk soil	0	0.82a	1.98a	1.13a	2.10a	2.96a	0.37a
	7	0.63a	2.55a	1.95a	2.79a	3.28a	0.21a
	14	0.49a	1.54a	1.00a	1.74a	2.32a	0.19a
	28	0.97a	2.56a	1.53a	3.26a	3.91a	0.41a

Means ($n = 4$) followed by different letters are significantly different at $P \leqslant 0.05$ within each mesh core treatment for each microbial group, simulation-based t-test.

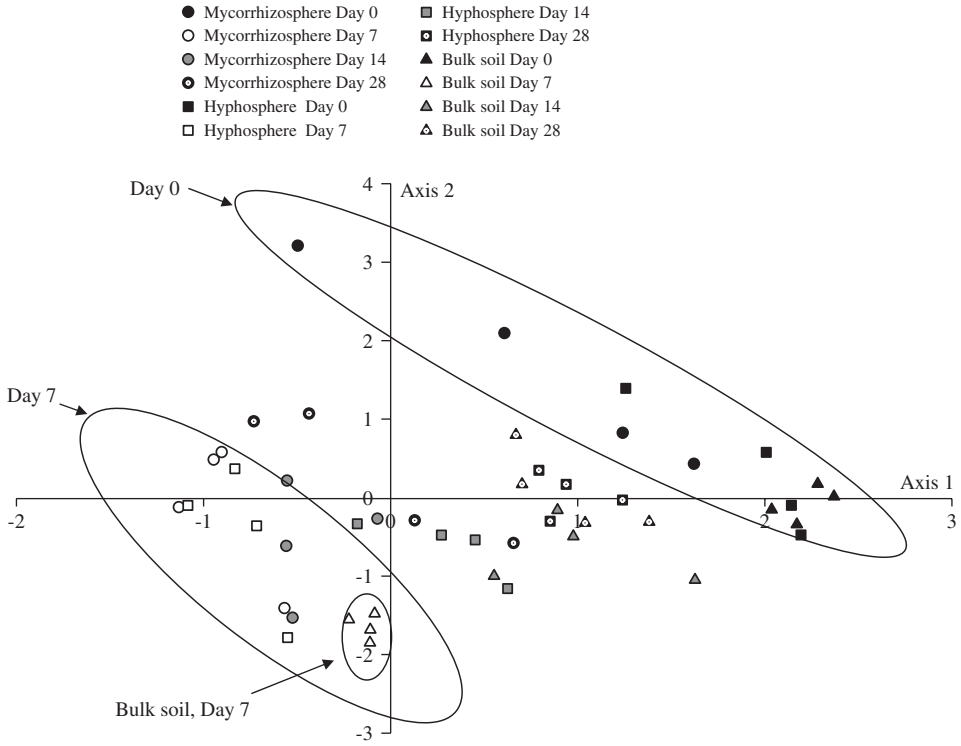

Fig. 4. Ordination biplot of soil microbial community structure within each mesh core treatment based on fatty acid composition. Each point is a single observation. Axes 1 and 2 represent 37.5% and 18.9% of the variation in the PLFA profiles, respectively.

associated with sampling date, suggesting that time was the most significant factor separating the samples (Fig. 4). Before addition of the cover crop litter to the mesh cores, on day 0, most samples grouped to the right side of axis 1. After addition of the cover crop litter, on day 7, all samples grouped to the left side of axis 1. On days 14 and 28, most samples returned to the right side of the first axis, closer to the day 0 samples. Within the clusters of days 0 and 7 samples, those from the bulk soil treatment tended to group together and apart from those of the mycorrhizosphere and hyphosphere treatments, especially on day 7. Samples from the mycorrhizosphere and hyphosphere treatments grouped together on days 0 and 7, making it difficult to distinguish them.

4. Discussion

4.1. ^{15}N uptake

We found that AM fungal hyphae helped grapevines take up ^{15}N from the cover crop litter, based on detection of significantly higher concentrations of ^{15}N among grapevines in the hyphosphere treatment than in the bulk soil treatment. Our evidence suggests that the additional ^{15}N in grapevines in the hyphosphere treatment was translocated by AM fungal hyphae that crossed the mesh screen and grew within the mesh cores to access the cover crop litter. These results are consistent with those of past research that relied on fine mesh screens to exclude roots and allow access by hyphae, which demonstrated that AM fungal hyphae take up and transport N to hosts from inorganic N sources (Ames et al., 1983; Johansen et al., 1992, 1994; Mäder et al., 2000) and organic N sources (Ames et al., 1983; Hawkins et al., 2000; Hodge et al., 2001).

Compared to uptake of ^{15}N by mycorrhizal roots in the mycorrhizosphere treatment, the contribution of AM fungal hyphae alone, in the hyphosphere treatment, was extremely low. By day 28, grapevines in the mycorrhizosphere treatment took up 15.6% of cover crop litter N, while those in the hyphosphere treatment took up 1.1%. The relatively minor contribution of AM fungal hyphae to ^{15}N uptake may be due to the fact that the grapevines were grown in nutrient-sufficient soil and were fertilized regularly. Johansen et al. (1994) showed that lower concentrations of N fertilizer were associated with increases in AM fungi-mediated transport of ^{15}N to host plants, suggesting that when N supply in the rhizosphere is deficient, AM fungal hyphae may play more important roles in transporting N to host plants. The contributions of AM fungi to host N nutrition may also depend on factors other than soil N content, such as the form of N in the soil (Ames et al., 1983). The significance of AM fungal contributions to host N nutrition varies widely among published reports, from 0.2% to 50% (Johansen et al., 1994; Hawkins and George, 1999; Hawkins et al., 2000; Mäder et al., 2000; Hodge et al., 2001). Most of these studies used soluble inorganic N or amino acids as added N sources, except the study by Hodge et al. (2001), who found that mycorrhizal *Plantago lanceolata* captured approximately 10% of ^{15}N from grass litter through AM fungal hyphae.

Our results demonstrate that grapevine fine roots play a dominant role in N uptake from litter when mycorrhizal roots and extraradical hyphae are both present. These findings suggest that while AM fungi can supplement nutrient uptake, healthy root growth is the prerequisite for a productive grapevine. Using vineyard management practices that foster root growth, such as planting vines in soil with adequate texture and structure, and irrigating vines during periods of rapid root growth, will likely have greater effects on uptake of soil-derived nutrients than adopting practices that focus solely on enhancing populations of AM fungi, such as the application of AM fungal inoculants to vineyard soil. It is important to note that the grapevines in our study were grown in nutrient-sufficient soil. Grapevines planted in marginal soils, an increasingly popular practice in the hillsides of the north-coastal grape-growing region of California (including Napa and Sonoma Counties), may rely more heavily on AM fungi for nutrient uptake than grapevines grown in nutrient-sufficient soils.

We expect that in the field, the contributions of grapevine roots and AM fungal hyphae to N uptake from cover crops is likely lower than we measured in our greenhouse study. Our design was intended to maximize litter decomposition and access to litter by roots and/or hyphae; hence the use of a leguminous cover crop and uniform incorporation of ground cover crop litter into the upper 20 cm of soil. In a tilled vineyard, fresh cover crop plants are often shallowly incorporated into the upper 10–15 cm of soil in the area between vineyard rows, where grapevine fine roots are sparse (Cheng and Baumgartner, 2005). Indeed, Patrick et al. (2004) found that the proportion of grapevine leaf N derived from a ^{15}N-labeled, leguminous cover crop was only 0.28% by the end of the growing season.

We assume the presence of extraradical hyphae inside the mesh cores of our hyphosphere treatment, based on high mycorrhizal colonization of grapevine roots and based on our finding of increased ^{15}N uptake among grapevines in the hyphosphere treatment, relative to the bulk soil treatment. Direct quantification of extraradical hyphae of AM fungi is challenging, due to a lack of suitable methods. AM hyphal length has been determined by visual discrimination from nonmycorrhizal hyphae or by comparing total hyphal length counts between inoculated plants and nonmycorrhizal controls (Jakobsen et al., 1992; Drew et al., 2003). However, the hyphal extraction process has some limitations. First, it destroys fine structure of hyphae and hyphal links. Second, it cannot easily recover the distal portions of the hyphae, which are more finely branched and intimately attached to the soil (Leake et al., 2004). Qualitative investigation of extraradical hyphae is possible. For example, previous studies that used fine mesh to exclude roots and allow AM hyphae to access various soil treatments have confirmed the presence or absence of extraradical hyphae by visual observation under the microscope (Jakobsen et al., 1992; Hodge et al., 2001). However, extraradical hyphae, especially the more narrow branches off the main hyphae, are morphologically similar to nonmycorrhizal fungi that are likely to be present in field soil (Smith and Read, 1997).

Our mesh cores were based on the design of Johnson et al. (2001), who constructed a core that could be left intact or rotated to allow or restrict access of hyphae. For the hyphosphere and bulk soil treatments, our modifications to their design included finer mesh and inclusion of an air gap between the two layers of mesh, to minimize mass flow and diffusion of ^{15}N. Despite this precautionary measure, we

found evidence of mass flow and diffusion. ^{15}N enrichment of soil outside the cores significantly increased throughout the experiment, starting at day 7, demonstrating that the longer the label remained in the cores, the easier mineralized ^{15}N moved out of the cores. We anticipated mass flow and diffusion in the mycorrhizosphere treatment, in which mesh size had to be large enough to allow access by roots. Indeed, soil outside the cores in the mycorrhizosphere treatment had higher ^{15}N enrichment than the other two treatments, albeit at concentrations that were likely insufficient (0.002–0.004% ^{15}N at% excess) to account for the higher ^{15}N uptake by grapevines in this treatment. Higher ^{15}N enrichment outside the cores in the mycorrhizosphere treatment may be due, in part, to uptake of ^{15}N by roots inside the cores and subsequent release outside the cores as a component of organic acids.

Our mesh cores have utility for evaluating the roles of mycorrhizae in host nutrition and for characterizing interactions between extraradical hyphae and other soil microbes. A modification that would accommodate more complex research questions includes incorporation of a layer of material that collects extraradical hyphae that span the mesh. Such a design would allow for quantification of extraradical hyphae and identification of AM fungal taxa.

4.2. Soil microbial community and cover crop decomposition

Our finding of similar peaks in total microbial biomass, after addition of the cover crop litter, in the mycorrhizosphere and hyphosphere treatments suggests that extraradical hyphae alone are capable of supporting a community of soil microbes within the mycorrhizosphere. Additional evidence in support of this hypothesis comes from correspondence analysis of the PLFA fingerprints, which grouped day 7 samples from the mycorrhizosphere and hyphosphere treatments together and apart from those of the bulk soil treatment. Extraradical hyphae can directly acquire recent photosynthate from host plants (Johnson et al., 2002) and a large proportion of extraradical hyphae turn over very quickly (Staddon et al., 2003). Therefore, we might expect that AM fungal hyphae serve as an important food source for other soil microbes. The fact that the microbial biomass in the bulk soil treatment, in which hyphae were not allowed to penetrate the mesh cores, did not respond to addition of cover crop litter to the mesh cores, is consistent with this expectation. Our findings contribute to the scant literature on interactions between AM fungi and other soil microbes (for example, Olsson et al., 1996; Amora-Lazcano et al., 1998; Edwards et al., 1998), and provide evidence that AM fungal hyphae may play an important role in supporting other soil microbes within the mycorrhizosphere.

We found that soil microbial biomass declined after the peaks on day 7, which is an observation that has also been made by other researchers (Wyland et al., 1996; Lundquist et al., 1999). Although microbial biomass responded to addition of cover crop litter in the hyphosphere treatment, as did microbial biomass in the mycorrhizosphere treatment, the hyphosphere did not sustain high levels of microbial biomass. Differences between the mycorrhizosphere and hyphosphere treatments in initial soil microbial biomass may explain the eventual decline to day 0 levels that were detected in the hyphosphere treatment after day 7. The extraradical hyphae alone within the mesh cores of the hyphosphere treatment likely supported a smaller amount of soil microbial biomass than the combination of roots and hyphae within the mesh cores of the mycorrhizosphere treatment. Given that soil microbes turn over quickly, it is possible that the biomass decreased to its original level in the hyphosphere treatment after the supply of readily available nutrients in the cover crop litter was utilized.

Although the initial increase in soil microbial biomass with addition of the cover crop litter was greater in the mycorrhizosphere and hyphosphere treatments than in the bulk soil treatment, ^{15}N and ^{13}C loss from the cover crop litter was not significantly different among the treatments. Unlike ectomycorrhizas and ericoid mycorrhizas, which have been shown to acquire N directly from organic materials (Abuzinadah and Read, 1989; Leake and Read, 1991; Cairney and Burke, 1998), AM fungi are believed to have no saprotrophic capabilities (Smith and Read, 1997). Evidence that contradicts this belief was published by Hodge et al. (2001), who showed that an AM fungal species, *Glomus hoi*, accelerated decomposition of complex organic materials. It is possible that the AM fungi in our study, which were cultured from grapevine roots, may not have the saprotrophic capabilities necessary to decompose cover crop litter.

There are contradictory reports about the effects of live roots on decomposition of organic matter (Reid and Goss, 1982; Sparling et al., 1982; Cheng and Coleman, 1990; Nicolardot et al., 1995; Hodge et al., 1998; Paré et al., 2000). Numerous plant and soil factors, such as plant species and phenology (Fu et al., 2002; Cheng et al., 2003), and soil mineral nutrition (Cheng and Coleman, 1990; Ehrenfeld et al., 1997), influence rhizosphere effects on organic matter decomposition. The small root biomass that grew in the mesh cores of the mycorrhizosphere treatment, 0.6 g, may not have been sufficient to bring about the associated rhizosphere effects on litter decomposition, especially in the nutrient-sufficient media.

Fatty acid 16:1ω5c has been used as a biomarker to estimate AM fungal biomass (Olsson et al., 1995, 1998; Olsson and Wilhelmsson, 2000). As expected, we found higher concentrations of 16:1ω5c in the mycorrhizosphere and hyphosphere treatments than in the bulk soil treatment. However, our finding that concentrations of 16:1ω5c responded to addition of cover crop litter over time suggest that 16:1ω5c may represent soil microbes other than AM fungi, a conclusion that other authors have come to, given that 16:1ω5c has also been found in bacteria (Nichols et al., 1986; Olsson et al., 1997). Additional evidence in support of this hypothesis is the high level of

16:1ω5c we measured in our growth medium, relative to soil collected from within the three mesh core treatments. Residual AM spores and bacteria in the field soil could have been the source of 16:1ω5c in the growth medium. Background levels of 16:1ω5c, the proportion not derived from AM fungi, have been estimated to range from 30% to 60% of total levels of this fatty acid in some studies (Olsson et al., 1995, 1997, 1998). Although 16:1ω5c has been found to be the dominant fatty acid in some AM fungal spores (Madan et al., 2002), it should be used with caution to quantify AM fungal biomass in natural communities of AM fungi and other soil microbes.

4.3. Future work

Extraradical hyphae constitute as much as 20–30% of the microbial biomass, and their length is typically one to two orders of magnitude higher than that of mycorrhizal roots (Leake et al., 2004). Their ability to form extensive extraradical hyphal networks among roots and root systems (Francis and Read, 1984; Newman et al., 1994; He et al., 2003) makes extraradical hyphae uniquely adapted to provide an important pathway for nutrient and C movement in the soil zone beyond the rhizosphere. Future work is needed to investigate the multifunctional roles of extraradical hyphae in sustainable agriculture and ecosystem function.

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